

# SEQUENCE LISTING

<110> Pastan, Ira H.  
Ho, Mitchell  
Bang, Sook-Hee  
The Government of the United States  
as represented by The Secretary of the  
Department of Health and Human Services

<120> Mutated Anti-CD22 Antibodies and Immunoconjugates

<130> 015280-500100US

<140> US 10/580,635

<141> 2006-05-25

<150> US 60/525,371

<151> 2003-11-25

<150> WO PCT/US04/39617

<151> 2004-11-24

<160> 30

<170> PatentIn Ver. 2.1

<210> 1

<211> 321

<212> DNA

<213> Mus sp.

<220>

<223> RFB4 mouse IgG1 anti-human CD22 monoclonal  
antibody light chain variable region (VL)

<220>

<221> CDS

<222> (1)..(321)

<223> RFB4 mouse IgG1 anti-human CD22 monoclonal  
antibody light chain variable region (VL)

<400> 1

gat atc cag atg acc cag act aca tcc tcc ctg tct gcc tct ctg gga	48
Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly	
1 5 10 15	
gac aga gtc acc att agt tgc agg gca agt cag gac att agc aat tat	96
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr	
20 25 30	
tta aac tgg tat cag cag aaa cca gat gga act gtt aaa ctc ctg atc	144
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile	
35 40 45	
tac tac aca tca ata tta cac tca gga gtc cca tca agg ttc agt ggc	192
Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly	
50 55 60	
agt ggg tct gga aca gat tat tct ctc acc att agc aac ctg gag caa	240
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln	
65 70 75 80	

gaa gat ttt gcc act tac ttt tgc caa cag ggt aat acg ctt ccg tgg 288  
 Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp  
                   85                  90                  95

acg ttc ggt gga ggc acc aag ctg gaa atc aaa 321  
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
                   100                  105

<210> 2  
 <211> 107  
 <212> PRT  
 <213> Mus sp.

<220>  
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal  
           antibody light chain variable region (VL)

<400> 2  
 Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly  
   1                  5                  10                  15  
 Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr  
                   20                  25                  30  
 Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile  
                   35                  40                  45  
 Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly  
           50                  55                  60  
 Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln  
   65                  70                  75                  80  
 Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp  
                   85                  90                  95  
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
                   100                  105

<210> 3  
 <211> 369  
 <212> DNA  
 <213> Mus sp.

<220>  
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal  
           antibody heavy chain variable region (VH)

<220>  
 <221> CDS  
 <222> (1)..(369)  
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal  
           antibody heavy chain variable region (VH)

<400> 3  
 gaa gtg cag ctg gtg gag tct ggg gga ggc tta gtg aag cct gga ggg 48  
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly  
   1                  5                  10                  15

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tcc ctg aaa ctc tcc tgt gca gcc tct gga ttc gct ttc agt atc tat 96
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile Tyr
                20                      25                      30

gac atg tct tgg gtt cgc cag act ccg gag aag agg ctg gag tgg gtc 144
Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
                35                      40                      45

gca tac att agt agt ggt ggt ggt acc acc tac tat cca gac act gtg 192
Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val
                50                      55                      60

aag ggc cga ttc acc atc tcc aga gac aat gcc aag aac acc ctg tac 240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
                65                      70                      75                      80

ctg caa atg agc agt ctg aag tct gag gac aca gcc atg tat tac tgt 288
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
                85                      90                      95

gca aga cat agt ggc tac ggt agt agc tac ggg gtt ttg ttt gct tac 336
Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr
                100                      105                      110

tgg ggc caa ggg act ctg gtc act gtc tct gca 369
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
                115                      120

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<210> 4  
 <211> 123  
 <212> PRT  
 <213> Mus sp.

<220>  
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal  
 antibody heavy chain variable region (VH)

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<400> 4
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
  1                      5                      10                      15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile Tyr
                20                      25                      30

Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
                35                      40                      45

Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val
                50                      55                      60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
                65                      70                      75                      80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
                85                      90                      95

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Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr  
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala  
115 120

<210> 5  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:carboxyl  
terminal fragment binding KDEL recycling receptor  
for transport of construct into cytosol from  
endoplasmic reticulum

<400> 5  
Lys Asp Glu Leu  
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<210> 6  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:carboxyl  
terminal fragment binding KDEL recycling receptor  
for transport of construct into cytosol from  
endoplasmic reticulum

<400> 6  
Arg Glu Asp Leu  
1

<210> 7  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
light chain (VL) complementarity determining  
region 1 (CDR1)

<400> 7  
Gln Asp Ile His Gly Tyr  
1 5

<210> 8  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
light chain (VL) complementarity determining  
region 1 (CDR1)

<400> 8  
Gln Asp Ile Gly Arg Tyr  
1 5

<210> 9  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
light chain (VL) complementarity determining  
region 1 (CDR1)

<400> 9  
Gln Asp Ile Arg Gly Tyr  
1 5

<210> 10  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
light chain (VL) complementarity determining  
region 1 (CDR1)

<400> 10  
Gln Asp Ile Ala Arg Tyr  
1 5

<210> 11  
<211> 3  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
light chain (VL) complementarity determining  
region 2 (CDR2)

<400> 11  
Tyr Thr Ser  
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<210> 12  
<211> 9  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
light chain (VL) complementarity determining  
region 3 (CDR3)

<400> 12  
Gln Gln Gly Asn Thr Leu Pro Trp Thr  
1 5

<210> 13  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
heavy chain (VH) complementarity determining  
region 1 (CDR1)

<400> 13  
Gly Phe Ala Phe Ser Ile Tyr Asp  
1 5

<210> 14  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
heavy chain (VH) complementarity determining  
region 2 (CDR2)

<400> 14  
Ile Ser Ser Gly Gly Gly Thr Thr  
1 5

<210> 15  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:RFB4 variable  
heavy chain (VH) complementarity determining  
region 2 (CDR2)

<400> 15  
Ala Phe His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr  
1 5 10 15

<210> 16  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:RFB4 variable  
heavy chain (VH) complementarity determining  
region 3 (CDR3)

<400> 16

Ala Phe His Ser Gly Tyr Gly Thr His Trp Gly Val Leu Phe Ala Tyr  
1 5 10 15

<210> 17

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:RFB4 variable  
heavy chain (VH) complementarity determining  
region 3 (CDR3)

<400> 17

Ala Phe His Ser Gly Tyr Gly Tyr Asn Trp Gly Val Leu Phe Ala Tyr  
1 5 10 15

<210> 18

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:RFB4 variable  
heavy chain (VH) complementarity determining  
region 3 (CDR3)

<400> 18

Ala Phe His Ser Gly Tyr Gly Thr Thr Trp Gly Val Leu Phe Ala Tyr  
1 5 10 15

<210> 19

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:RFB4 variable  
heavy chain (VH) complementarity determining  
region 3 (CDR3)

<400> 19

Ala Phe His Ser Gly Tyr Gly Ser Thr Tyr Gly Val Leu Phe Ala Tyr  
1 5 10 15

<210> 20

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:mutated RFB4 VL  
chain

<400> 20

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly  
1 5 10 15

Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile His Gly Tyr  
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile  
35 40 45

Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp  
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> 21

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:mutated RFB4 VH  
chain

<400> 21

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly  
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile Tyr  
20 25 30

Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val  
35 40 45

Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr  
65 70 75 80

Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys  
85 90 95

Ala Arg His Ser Gly Tyr Gly Thr His Trp Gly Val Leu Phe Ala Tyr  
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala  
115 120



<210> 22  
 <211> 345  
 <212> PRT  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Pseudomonas  
 exotoxin A cytotoxic fragment PE38 translocating  
 and ADP ribosylating domains

<400> 22

Gly	Gly	Ser	Leu	Ala	Ala	Leu	Thr	Ala	His	Gln	Ala	Cys	His	Leu	Pro
1				5					10					15	
Leu	Glu	Thr	Phe	Thr	Arg	His	Arg	Gln	Pro	Arg	Gly	Trp	Glu	Gln	Leu
			20					25					30		
Glu	Gln	Cys	Gly	Tyr	Pro	Val	Gln	Arg	Leu	Val	Ala	Leu	Tyr	Leu	Ala
		35					40					45			
Ala	Arg	Leu	Ser	Trp	Asn	Gln	Val	Asp	Gln	Val	Ile	Arg	Asn	Ala	Leu
		50				55					60				
Ala	Ser	Pro	Gly	Ser	Gly	Gly	Asp	Leu	Gly	Glu	Ala	Ile	Arg	Glu	Gln
65					70					75					80
Pro	Glu	Gln	Ala	Arg	Leu	Ala	Leu	Thr	Leu	Ala	Ala	Ala	Glu	Ser	Glu
				85					90					95	
Arg	Phe	Val	Arg	Gln	Gly	Thr	Gly	Asn	Asp	Glu	Ala	Gly	Ala	Ala	Asn
			100					105					110		
Gly	Pro	Ala	Asp	Ser	Gly	Asp	Ala	Leu	Leu	Glu	Arg	Asn	Tyr	Pro	Thr
		115					120					125			
Gly	Ala	Glu	Phe	Leu	Gly	Asp	Gly	Gly	Asp	Val	Ser	Phe	Ser	Thr	Arg
		130				135					140				
Gly	Thr	Gln	Asn	Trp	Thr	Val	Glu	Arg	Leu	Leu	Gln	Ala	His	Arg	Gln
145					150					155					160
Leu	Glu	Glu	Arg	Gly	Tyr	Val	Phe	Val	Gly	Tyr	His	Gly	Thr	Phe	Leu
				165					170					175	
Glu	Ala	Ala	Gln	Ser	Ile	Val	Phe	Gly	Gly	Val	Arg	Ala	Arg	Ser	Gln
			180					185					190		
Asp	Leu	Asp	Ala	Ile	Trp	Arg	Gly	Phe	Tyr	Ile	Ala	Gly	Asp	Pro	Ala
		195					200					205			
Leu	Ala	Tyr	Gly	Tyr	Ala	Gln	Asp	Gln	Glu	Pro	Asp	Ala	Arg	Gly	Arg
		210				215					220				
Ile	Arg	Asn	Gly	Ala	Leu	Leu	Arg	Val	Tyr	Val	Pro	Arg	Ser	Ser	Leu
225					230					235					240
Pro	Gly	Phe	Tyr	Arg	Thr	Ser	Leu	Thr	Leu	Ala	Ala	Pro	Glu	Ala	Ala
				245					250					255	
Gly	Glu	Val	Glu	Arg	Leu	Ile	Gly	His	Pro	Leu	Pro	Leu	Arg	Leu	Asp
			260					265					270		

Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu  
275 280 285

Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile Pro Ser Ala Ile Pro  
290 295 300

Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro  
305 310 315 320

Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp Tyr Ala Ser Gln Pro  
325 330 335

Gly Lys Pro Pro Arg Glu Asp Leu Lys  
340 345

<210> 23  
<211> 345  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Pseudomonas exotoxin A  
cytotoxic fragment PE38 translocating and ADP ribosylating  
domains with Arg at position 222 of PE38 (position 490 of  
Pseudomonas exotoxin A) mutated to Ala

<400> 23  
Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro  
1 5 10 15

Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu  
20 25 30

Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala  
35 40 45

Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu  
50 55 60

Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln  
65 70 75 80

Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu  
85 90 95

Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn  
100 105 110

Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr  
115 120 125

Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg  
130 135 140

Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu Gln Ala His Arg Gln  
145 150 155 160

Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr His Gly Thr Phe Leu  
165 170 175

Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val Arg Ala Arg Ser Gln  
 180 185 190  
 Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile Ala Gly Asp Pro Ala  
 195 200 205  
 Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro Asp Ala Ala Gly Arg  
 210 215 220  
 Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val Pro Arg Ser Ser Leu  
 225 230 235 240  
 Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala Ala Pro Glu Ala Ala  
 245 250 255  
 Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu Pro Leu Arg Leu Asp  
 260 265 270  
 Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu  
 275 280 285  
 Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile Pro Ser Ala Ile Pro  
 290 295 300  
 Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro  
 305 310 315 320  
 Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp Tyr Ala Ser Gln Pro  
 325 330 335  
 Gly Lys Pro Pro Arg Glu Asp Leu Lys  
 340 345

<210> 24  
 <211> 613  
 <212> PRT  
 <213> Pseudomonas sp.

<220>  
 <223> Pseudomonas exotoxin A

<400> 24  
 Ala Glu Glu Ala Phe Asp Leu Trp Asn Glu Cys Ala Lys Ala Cys Val  
 1 5 10 15  
 Leu Asp Leu Lys Asp Gly Val Arg Ser Ser Arg Met Ser Val Asp Pro  
 20 25 30  
 Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met Val  
 35 40 45  
 Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala Leu  
 50 55 60  
 Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val Glu  
 65 70 75 80  
 Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly Ser  
 85 90 95

Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser Asn  
 100 105 110  
 Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser His  
 115 120 125  
 Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala Lys  
 130 135 140  
 Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn Glu  
 145 150 155 160  
 Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val Met  
 165 170 175  
 Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala Ser  
 180 185 190  
 Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn Tyr  
 195 200 205  
 Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys Ile  
 210 215 220  
 Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile Lys  
 225 230 235 240  
 Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser Leu  
 245 250 255  
 Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr Phe  
 260 265 270  
 Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys Gly  
 275 280 285  
 Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu Ser  
 290 295 300  
 Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro Gly  
 305 310 315 320  
 Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln Ala  
 325 330 335  
 Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu Arg Phe Val Arg  
 340 345 350  
 Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn Ala Asp Val Val  
 355 360 365  
 Ser Leu Thr Cys Pro Val Ala Ala Gly Glu Cys Ala Gly Pro Ala Asp  
 370 375 380  
 Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu Phe  
 385 390 395 400  
 Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Thr Gln Asn  
 405 410 415

Trp Thr Val Glu Arg Leu Leu Gln Ala His Arg Gln Leu Glu Glu Arg  
 420 425 430  
 Gly Tyr Val Phe Val Gly Tyr His Gly Thr Phe Leu Glu Ala Ala Gln  
 435 440 445  
 Ser Ile Val Phe Gly Gly Val Arg Ala Arg Ser Gln Asp Leu Asp Ala  
 450 455 460  
 Ile Trp Arg Gly Phe Tyr Ile Ala Gly Asp Pro Ala Leu Ala Tyr Gly  
 465 470 475 480  
 Tyr Ala Gln Asp Gln Glu Pro Asp Ala Arg Gly Arg Ile Arg Asn Gly  
 485 490 495  
 Ala Leu Leu Arg Val Tyr Val Pro Arg Ser Ser Leu Pro Gly Phe Tyr  
 500 505 510  
 Arg Thr Ser Leu Thr Leu Ala Ala Pro Glu Ala Ala Gly Glu Val Glu  
 515 520 525  
 Arg Leu Ile Gly His Pro Leu Pro Leu Arg Leu Asp Ala Ile Thr Gly  
 530 535 540  
 Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu Gly Trp Pro Leu  
 545 550 555 560  
 Ala Glu Arg Thr Val Val Ile Pro Ser Ala Ile Pro Thr Asp Pro Arg  
 565 570 575  
 Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro Asp Lys Glu Gln  
 580 585 590  
 Ala Ile Ser Ala Leu Pro Asp Tyr Ala Ser Gln Pro Gly Lys Pro Pro  
 595 600 605  
 Arg Glu Asp Leu Lys  
 610

<210> 25  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:two-step  
 overlap PCR upstream mutagenic primer A

<400> 25  
 gaacccgacg cagccggccg tatccgcaac

30

<210> 26  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:two-step  
 overlap PCR downstream mutagenic primer B

<400> 26  
 gttgcggata cggccggctg cgtcgggttc 30

<210> 27  
 <211> 26  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:two-step  
 overlap PCR mutagenic primer C

<400> 27  
 gctgtcgtgg aaccaggtcg accagg 26

<210> 28  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:two-step  
 overlap PCR mutagenic primer D

<400> 28  
 ctttgtagc agccgaattc atattcgat 29

<210> 29  
 <211> 4  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:RFB4 VH chain  
 CDR3 in which "SSY" mutated to "THW"

<400> 29  
 Gly Thr His Trp  
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<210> 30  
 <211> 5  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:carboxyl  
 terminal native sequence binding KDEL recycling  
 receptor for transport of construct into cytosol  
 from endoplasmic reticulum

<400> 30  
 Arg Glu Asp Leu Lys  
 1 5